Early life bereavement and childhood cancer: a nationwide follow-up study in two countries

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Title: Early life bereavement and childhood cancer: a nationwide follow-up study in two countries

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Abstract

Objective: Childhood cancer is a leading cause of child deaths in affluent countries but little is known about its aetiology. Psychological stress has been suggested to be associated with cancer in adults; whether this is also seen in childhood cancer is largely unknown. We investigated the association between bereavement as an indicator of childhood stress exposure and childhood cancer, using data from Danish and Swedish national registers.

Design: Population-based cohort study.

Setting: Denmark and Sweden.

Participants: All live-born children born in Denmark between 1968 and 2007 (N=2,729,308) and in Sweden between 1973 and 2006 (N=3,395,166) were included in this study. Exposure was bereavement by the death of a close relative before 15 years of age. Follow up started from birth and ended at the first of the following: date of a cancer diagnosis, death, emigration, day before their 15th birthday or end of follow up (2007 in Denmark, 2006 in Sweden).

Outcome measures: Rates and hazard ratios for all childhood cancers and specific childhood cancers.

Results: A total of 1,505,938 (24.5%) children experienced bereavement. There were 9823 who received a cancer diagnosis. Exposed children had a small (10%) increased risk of childhood cancer (hazard ratio (HR): 1.10; 95% confidence interval (CI) 1.04-1.17). For specific cancers, a significant association was seen only for central nervous system tumours (HR: 1.14; 95% CI: 1.02-1.28).

Conclusions: Our data suggests psychological stress in early life is associated with a small increased risk of childhood cancer.

Key words: bereavement, psychological stress, childhood cancer, follow up, risk factor

Article summary

Article focus: (up to 3 bullets on the research questions or hypotheses addressed)

- There is limited information on the aetiology of childhood cancers and psychological stress may play a role.
- We investigated the association between psychological stress following bereavement by the death of a close relative early in life and subsequent childhood cancer.

Key messages: (up to 3 bullets on the key messages/significance)

- A small (10%) increase in the risk of childhood cancer was seen among those who experienced the death of a close relative.
- The association between early life stress and childhood cancer was small, but adds to our understanding of the causes of childhood cancers.

Strengths and limitations

- The study utilizes nationwide registers in two countries, which contain data of high quality. Such a large sample size is important for such research due to the rareness of childhood cancer.
- There are probably other sources of stress on which we do not have information, but the death of a close relative is considered to be one of the most stressful experiences, which will provide a good exposure contrast.
- The limited information on the aetiology of childhood cancers means that confounder control may be incomplete in this study.

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Introduction

Childhood cancer is a leading cause of child deaths in affluent societies [1,2]. Almost half of childhood cancers are diagnosed before 5 years of age [2], highlighting the importance of identifying early life risk factors for developing prevention strategies [2,3] but, in comparison to adult cancers, known risk factors are few, except for radiation [4,5]. Additionally, heterogeneity and rareness of childhood cancers make investigation in populations challenging.

Studies in adults have reported increased cancer risks following psychological stress [6,7]. Psychological stress activates the nervous system and the hypothalamic-pituitary-adrenal axis, leading to release of hormones such as glucocorticoids and norepinephrine. Research has shown stress and the subsequent hormonal dysfunction can lead to impairment of DNA repair [8] and suppression of the immune response [9]. Additionally, stress may lead to epigenetic silencing: altering DNA methylation and histone acetylation [10], which are important during tumour development [11,12].

Little is known, however, about the effect of stress in early postnatal life on the risk of childhood cancers and the effect of stress is potentially a much more complex exposure in children than in adults. Firstly, children have immature body systems; growth and differentiation of their organs can be disrupted [13], potentially increasing susceptibility to environmental exposures. Through its effects on immune function, stress may increase susceptibility to infections, which have also been linked to certain childhood cancers [14-17]. However, “resilience to adversity” [18] might imply that stress may not lead to the same hormonal and immunological disturbances observed in adults. Our previous work on psychological stress in adults uses bereavement, considered to be

one of the most stressful experiences [19], as an indicator for psychological stress [6,20]. Just as adults may engage in risk behaviors following stress [21], children may be exposed to additional risks (such as passive smoking, poor diet, physical inactivity, or premature cessation of breast feeding) if parents altered their own lifestyle due to bereavement, which in turn impacts their children. However, the death of a relative may have further consequences for children. Other changes may also occur following bereavement, including reduced economic resources, changes in care [22] or a change in parents' ability to fulfill parental roles due to their own grief [23]. Another difference is that young children may a lack of understanding about death [23], and therefore the experiences of psychological stress following bereavement may vary compared with adults and between age groups in childhood.

We investigated the association between bereavement in early life and the subsequent risk of childhood cancers. We hypothesized that that risks would be of a larger magnitude following the death of a close relative versus the death of other relatives, and sudden death versus other death [6,20]. In addition, we hypothesized that risks would vary with timing of the exposure, due to differences in awareness of loss [23] and susceptibility to changes in family structure [22,23].

Materials and Methods

Study participants and follow-up

This population-based cohort study used data from Danish and Swedish national registers and its design has been described elsewhere [24]. In brief, the unique civil personal registration number, which is assigned to all live-born children and new residents, was used to link information on birth, death, demographics, social data and various health outcomes from different national registers. Children of mothers with no personal registration number recorded were excluded as they could not be linked to their relatives (N=10,641; 0.17%). Additionally, children diagnosed with cancer within three months of birth were excluded, to remove cancers likely to have been prevalent at birth (N=348; 0.01%). The remaining study populations included 2,729,308 children from Denmark and 3,395,166 children from Sweden.

The exposure for the study is bereavement by the death of a close relative. Children born in Denmark from 1968 to 2007 and in Sweden from 1973 to 2006 were linked to their relatives (parents, siblings, mother's siblings and mother's parents) by their personal registration number, using the Danish Civil Registry System and the Swedish Multigeneration Register. Data on relatives' deaths were obtained from the Danish Civil Registry and the Swedish Cause of Death Registry. Follow up started at birth when all children were classified as 'unexposed'. Children would be categorized as 'exposed' when they experienced the death of a close relative, and afterwards contributed observation time for the exposed group. Follow up ended at the first of the following events: date of a cancer diagnosis, death, emigration, day before their 15th birthday or end of follow up (31st December 2006 in Sweden and 31st December 2007 in Denmark). Covariates were selected a priori according to previous literature: potential confounders at baseline (birth)

included maternal age (≤26 years, 27-30 years, >30 years), parity (1, 2, ≥3), multiplicity, maternal education level at birth (≤9 years, 10-14 years, >14 years), and maternal smoking during pregnancy. Data on covariates were obtained from the Medical Birth Registries, the Swedish Education Register and the Danish Integrated Database for Labour Market Research.

The data on cancer diagnoses was obtained from the Swedish and Danish National Cancer Registries [25,26]. The main outcomes of interest were all incident cancers (ICD-7 codes 104-205, ICD-10 codes C00-97) diagnosed up to the 15th year of age. Additionally, specific childhood cancer diagnoses previously suggested to be related to stress, hormones or immune status were considered, including testicular cancer (ICD-7 178, ICD-10 C62) [27], leukaemias (ICD-7 204, ICD-10 C91-95) [28,29] or lymphomas (Hodgkin's lymphoma (ICD-7 201, ICD-10 C81) and non-Hodgkin's lymphoma (ICD-7 200 and 202, ICD-10 C82-83)) [20,29,30]. As there is limited evidence on childhood cancer aetiology, we also included central nervous system (CNS) tumours (ICD-7 193, ICD-10 C70-72) and Wilms' tumour (ICD-7 180, ICD-10 C69.2).

Statistical analysis

Statistical analyses were carried out using Stata 11. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using the Cox regression model, accounting for some mothers having more than one child in the cohort by using robust estimation. Proportional-hazards assumption was evaluated using the *estat phtest* function, which tests the assumption on the basis of Schoenfeld residuals. The analyses were stratified by type of bereavement (the death of a parent/sibling, or of another relative), cause of death (unexpected death due to an accident, suicide or violence, or other death) and timing of the exposure (age at exposure 0-1 years, 2-5

years, 6-9 years or 10-14 years) [19,31,32]. Potential confounders like maternal age, parity and multiplicity were adjusted. Additionally, stratification was carried out by sex, country, birth weight and gestational age of the children. In sub-analyses, we also adjusted for smoking during pregnancy (available from 1991 onwards in Denmark and from 1983 onwards in Sweden) and maternal education at birth (available from 1980 onwards in Denmark and from 1973 onwards in Sweden), and birth year was added to adjust for calendar time. To check for the possibility of confounding by a genetic predisposition to cancer, the analyses were repeated following exclusion of children whose bereavement was caused by death of a parent from cancer. Additionally, analyses were carried out where children were moved to the exposed group three months after they experienced a death of a relative, to allow some time for a potential physiological effect of the bereavement. Finally, multiple imputations were carried out for missing covariates using Stata's *ice* command. Maternal origin, birth year, birth weight and gestational age were also included in the imputation model.

The study was approved by the Danish Data Protection Agency (j nr. 2008-41-2680), Scientific Ethics Committee of Central Jutland Region (VEK, sagnr. M-20100252) and the Research Ethic Committee (EPN) at the Karolinska Institute (Ref no. 2008/4:6).

Results

Of the 6,124,474 children, 1,505,938 (24.5%) experienced death of a relative during the follow up period of 71.9 million person years. In the cohort, 9823 children were diagnosed with cancer (incidence rate 13.7 per 100,000 person years); the most common being leukaemias (2882), cancers of the CNS (2546) and Wilms' tumours (606). Of children exposed to death of a relative, 1350 received a diagnosis of childhood cancer.

The characteristics of the study population are provided in Table 1. Children in the exposed group were more likely to have had low birth weight, to be of higher birth order and to be born to mothers who were older, of Nordic origin, with lower education levels and who more often reported smoking during pregnancy. Additionally, children in the exposed cohort were more likely to have a low Apgar score at five minutes (Table 1).

[INSERT TABLE 1 HERE]

The associations between bereavement and childhood cancer are displayed in Table 2. Compared with unexposed children, exposed children had a slightly increased cancer risk (HR: 1.10; 95% CI: 1.04-1.17). When splitting by type of relative, the association between parent/sibling death and childhood cancers was positive, but statistically insignificant. For more distant relatives the association was smaller, but statistically significant. The association was also significant for who experienced the death of a relative due to a disease and for those bereaved between 2-5 years of age (Table 2). Adding birth year, maternal smoking during pregnancy and maternal education at birth to the models did not alter the results (data not shown).

[INSERT TABLE 2 HERE]

When we excluded children whose exposure status changed following the death of a parent from cancer (n=31,737), the overall risk of childhood cancer related to loss of a relative was almost unchanged (HR: 1.09; 95% CI: 1.03-1.16); and if the death was due to a disease, the HR (95% CI) was 1.10 (1.04-1.18). Moving children to the exposed group three months after they experienced a death of a relative also gave similar results (HR: 1.09; 95% CI: 1.03-1.16). Following imputation of missing data, results were not greatly altered: the HR (95% CI) was 1.13 (1.07-1.20).

Stratified analyses did not show significant effect measure modification by sex, low (<2500g) versus normal (≥2500g) birth weight and preterm (<37 weeks) versus term (≥37 weeks) birth (data not displayed), but exposure groups became small for these analyses. Additionally, the data was stratified by country (Sweden versus Denmark). While confidence intervals for the HRs overlapped, the association between exposure and childhood cancer was larger for Sweden (HR: 1.12; 95% CI: 1.03-1.20) than for Denmark (HR: 1.07; 95% CI: 0.97-1.19).

The associations between bereavement and specific childhood cancers are shown in Table 3. Numbers of cases were particularly small for testicular cancer and Hodgkin's lymphoma, and for exposure subgroups; results from subgroups are therefore not displayed. Those exposed were observed to have a significantly increased risk for CNS tumours (HR: 1.14; 95% CI: 1.02-1.28). A positive association was also seen for leukemia (HR: 1.12; 95% CI: 1.00-1.26) (Table 3).

[INSERT TABLE 3 HERE]

Discussion

In this large population-based cohort study, a small but statistically significant overall increased risk of childhood cancer was observed among children exposed to bereavement due to the death of a family member. Exposure was also associated with CNS tumours and leukaemia. However, limited number of cases prevented us from obtaining informative estimates for most of specific childhood cancers.

The main methodological strength of the study is the use of the large, longitudinal, nationwide registers from the Denmark and Sweden. Data was collected prospectively and is of high quality, with almost complete follow up [33]. Although childhood cancers are a leading cause of childhood death, they are not very common, making ad hoc follow up studies rare in previous literature. The cancer registries have high levels of completeness [25,26]. Bereavement due to death of a relative provides a good indicator of stress, which is considered one of the most stressful events [19], irrespective of coping style [34].

A limitation is the uncertainty of induction and latency periods for childhood cancer. The main analysis was therefore repeated after considering start of exposure to be three months after they experienced the bereavement, allowing some time for a potential physiological effect of the bereavement. This did not provide different results. Compared with the unexposed group, there were fewer missing values for some co-variates in the exposed group. This may partly be due to a higher proportion of children born to mothers of Nordic origin in the exposed group (98.5% compared with 94.0%), as information for mothers of non-Nordic origin may be less complete. We did not have information on other stressful events, for example parental divorce or the death of a

caregiver. However those who experienced other causes of stress would be categorized as 'unexposed', and such misclassification would probably draw risk estimates towards the null.. Finally, despite a large sample size, case numbers for some specific cancers were small.

We have previously reported that mothers who experience the death of a child have increased cancer risk [6], and if mothers are exposed to bereavement during pregnancy, the risk of some childhood cancers in the offspring is increased [20]. Considering the differences in the effects of stress in children compared to adults, we hypothesized variation in the size of the association based on age at bereavement, as there may be little awareness of a loss at a very young age. Although a significant increase in risk was seen following bereavement between the ages of 2-5 years, there were no significant differences between age groups. One potential reason is that bereavement, particularly of a close relative, can be a long lasting stressor leading to allostatic load [35], and not limited to the period immediately after bereavement. Other studies looking at stressful life events and malignant disease in adults have produced mixed results: some found no association [36-41], while some have reported increased risks [6,7]. Most studies have included less severe stress exposures than loss of a close relative, and similar to our findings, positive associations have generally been of modest magnitude, indicating that if stress is a causal factor it is only one of many potential causes.

Although it is unlikely that bereavement increases risks for all childhood cancers, the observed associations suggest a role of for hormonal disturbance perhaps as a general promoter. The function of immune cells following a stressful exposure may impair the ability to detect and deal with cancerous cells and eliminate infections. For example, in adults stress may inhibit apoptosis

[9]. Bereavement during childhood has been described as a “tolerable stress”, which can be overcome with the right support, but may become “toxic” if unmanaged [42]. Thus, insufficient coping and limited social support may further lead to allostatic load prolonging the hormonal imbalance. Whether the associations reflect a direct causal effect of stress or an indirect effect, mediated by other changes (for example, diet, infections or family dynamics), or are a result of unadjusted confounding is not known. We do not know more than a small fraction of childhood cancer causes, making confounder adjustments far from complete. The ‘two-hit theory’ [43] and multi-step theory [44] of carcinogenesis suggest that at least two mutagenic hits are necessary for cancer development. Research has suggested that some childhood cancers, including leukaemias [45] and CNS tumours [46], are initiated in utero. If psychological stress does affect childhood cancer risk, bereavement may act as or facilitate the second ‘hit’. The need for exposure to a risk factor during gestation to initiate development of a childhood cancer and the vast number of other potential causes of ‘hits’ could explain the relatively small association seen in this study.

If stress has a causal relation with certain childhood cancers, it would be expected to vary with the intensity of stress. However, stress is a “highly individualistic experience” [47], which may make it difficult to consider a dose-response effect. The risk of childhood cancer was not higher in those who lost a parent or a sibling than in those who lost a more distant relative. Additionally, there were no differences in risk if the loss was sudden or due to a disease, but numbers become small for these sub-analyses. It is also difficult to hypothesize, especially in this age group, which would cause a greater level of stress: an unexpected loss or loss from a chronic disease. A long term effect may be more important, which follow either type of loss.

Our data suggests that psychological stress in early life is associated with an increased risk of some childhood cancers. Early life bereavement may also have long term effects on cancer risk. For example, epigenetic changes or impairment of DNA repair may reduce the body's ability to deal with the future carcinogenic exposures [8,12]. Inclusion of data from more countries or over a longer time period could provide greater power to better assess the association between stress and specific cancers. The association between early life stress and childhood cancer was small, but adds to our general understanding of the causes and development of childhood cancers.

Contributors: JL and JO conceived the research. NM analysed the data and wrote the first draft of the manuscript. NM, JO, SC, MG and JL contributed to data analysis, interpretation of results and critical revision of the manuscript.

Data sharing: The data is accessed via secure server at Statistics Denmark. We, the researchers, are unable to provide access to the raw data.

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Table 1. Descriptive statistics of the study population

	Exposed cohort (N = 1,426,013)	Unexposed cohort (N = 6,123,531)
Variables	**N (%)**	**N (%)**
Sex		
Male	773,690 (51)	3,143,231 (51)
Female	732,244 (49)	2,980,280 (49)
Missing	4 (<1)	20 (<1)
Birth weight [a]		
<2500g	68,125 (5)	232,387 (4)
≥2500g	1,369,759 (94)	4,938,970 (92)
Missing	22,344 (2)	215,793 (4)
Maternal age		
≤26	519,495 (35)	2,398,883 (39)
27-30	443,592 (30)	1,777,990 (29)
≥31	542,782 (36)	1,946,022 (32)
Missing	69 (<1)	636 (<1)
Gestational age [a]		
<37 weeks	82,407 (6)	82,407 (6)
≥37 weeks	1,351,860 (93)	1,351,860 (93)
Missing	25,961 (2)	25,961 (2)
Parity [b]		
1	582,558 (41)	2,648,077 (46)
2	519,142 (37)	2,030,259 (35)
≥3	305,963 (22)	1,059,912 (18)
Missing	806 (<1)	10,633 (<1)
Maternal education [c]		
Low, ≤9 years	701,579 (49)	2,306,817 (44)
Middle, 10-14 years	398,797 (28)	1,461,584 (28)
High, ≥15 years	296,247 (21)	1,046,192 (20)
Missing	44,454 (3)	442,534 (8)
Maternal smoking during pregnancy [d]		
Yes	222.980 (26)	703,266 (19)
No	572.800 (66)	2,674,088 (72)
Missing	69,925 (8)	315,934 (9)
Singleton [b]		
Yes	1,437,016 (97)	5,451,148 (95)
No	39,129 (3)	147,173 (3)
Missing	10,030 (1)	150,560 (3)
Apgar score at 5 minutes [a]		
Low, 0-6	13,960 (1)	52,667 (1)
Normal, 7-10	1,352,106 (93)	4,946,721 (92)
Missing	94,162 (6)	387,762 (7)
Country		
Denmark	663,820 (44)	2,728,862 (45)
Sweden	842,118 (56)	3,394,669 (55)
Maternal origin		
Nordic	1,482,681 (99)	5,758,361 (94)
Non-Nordic	19,532 (1)	333,804 (6)

Missing	3,725 (<1)	31,366 (1)

[a] available from 1978 in Denmark and from 1973 in Sweden; [b] available from 1973 in Denmark and from 1973 in Sweden; [c] available from 1980 in Denmark and from 1973 in Sweden; [d] available from 1991 in Denmark and from 1983 in Sweden

Table 2. Hazard ratios (HR) for all childhood cancers according to exposure status

Bereavement	Cancer cases (rate 1/100 000 person years)	Crude HR	Adjusted HR (95% CI) [a]
All exposed	1350 (13.88)	1.15	1.10 (1.04-1.17)
Type of deceased relative			
Parent/sibling	140 (14.44)	1.20	1.18 (0.99-1.41)
Other relatives	1210 (13.82)	1.14	1.09 (1.03-1.16)
Cause of death			
Unexpected	132 (12.83)	1.06	1.03 (0.87-1.23)
Other	1217 (14.07)	1.16	1.11 (1.04-1.19)
Age at exposure			
0-1 years	350 (14.88)	1.10	1.06 (0.95-1.18)
2-5 years	536 (13.88)	1.17	1.13 (1.03-1.23)
6-9 years	306 (12.48)	1.13	1.08 (0.95-1.21)
10-14 years	158 (14.88)	1.17	1.14 (0.99-1.39)
Unexposed	8473 (13.64)	1.0 (ref)	1.0 (ref)

[a] Adjusted for country, maternal characteristics at birth (maternal age and parity) and whether child was a twin. Reduced to 9382 failures (and 9381 for unexpected vs other) due to missing values for covariates and twin/singleton data availability from 1973.

Table 3. Hazard ratios (HR) for specific childhood cancers according to bereavement

Cancer		Cases (rate per 100,000 person years)	Crude HR	Adjusted HR (95% CI) [a]
Leukaemias	Unexposed	2522 (4.06)	1.0 (ref)	1.0 (ref)
	All Exposed	360 (3.70)	1.2	1.12 (1.00-1.26)
Hodgkin's Lymphoma	Unexposed	123 (0.20)	1.0 (ref)	1.0 (ref)
	All Exposed	43 (0.44)	1.29	1.17 (0.81-1.67)
Non-Hodgkin's Lymphoma	Unexposed	441 (0.71)	1.0 (ref)	1.0 (ref)
	All Exposed	68 (0.70)	1.03	0.99 (0.76-1.29)
CNS tumours	Unexposed	2160 (3.48)	1.0 (ref)	1.0 (ref)
	All Exposed	386 (3.97)	1.19	1.14 (1.02-1.28)
Wilms' tumour [b]	Unexposed	564 (0.91)	1.0 (ref)	1.0 (ref)
	All Exposed	42 (0.43)	0.98	0.93 (0.68-1.28)
Testicular cancer	Unexposed	50 (0.08)	1.0 (ref)	1.0 (ref)
	All Exposed	5 (0.05)	1.16	1.08 (0.41-2.88)

[a] Adjusted for country, maternal characteristics at birth (maternal age and parity) and whether child was a twin.
[b] Proportional hazards assumption not met by model for this cancer subtype.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9
Bias	9	Describe any efforts to address potential sources of bias	8-9
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	8-9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10, Table 2
		(*b*) Report category boundaries when continuous variables were categorized	8, Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11, Table 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	4

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Early life bereavement and childhood cancer: a nationwide follow-up study in two countries

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Title: Early life bereavement and childhood cancer: a nationwide follow-up study in two countries

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Key words: bereavement, psychological stress, childhood cancer, risk factor , follow up

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Abstract

Objective: Childhood cancer is a leading cause of child deaths in affluent countries but little is known about its aetiology. Psychological stress has been suggested to be associated with cancer in adults; whether this is also seen in childhood cancer is largely unknown. We investigated the association between bereavement as an indicator of severe childhood stress exposure and childhood cancer, using data from Danish and Swedish national registers.

Design: Population-based cohort study.

Setting: Denmark and Sweden.

Participants: All live-born children born in Denmark between 1968 and 2007 (N=2,729,308) and in Sweden between 1973 and 2006 (N=3,395,166) were included in this study. Exposure was bereavement by the death of a close relative before 15 years of age. Follow up started from birth and ended at the first of the following: date of a cancer diagnosis, death, emigration, day before their 15th birthday or end of follow up (2007 in Denmark, 2006 in Sweden).

Outcome measures: Rates and hazard ratios for all childhood cancers and specific childhood cancers.

Results: A total of 1,505,938 (24.5%) children experienced bereavement at some point during their childhood and 9823 were diagnosed with cancer before the age of 15 years. Exposed children had a small (10%) increased risk of childhood cancer (hazard ratio (HR): 1.10; 95% confidence interval (CI) 1.04-1.17). For specific cancers, a significant association was seen only for central nervous system tumours (HR: 1.14; 95% CI: 1.02-1.28).

Conclusions: Our data suggests psychological stress in early life is associated with a small increased risk of childhood cancer.

Key words: bereavement, psychological stress, childhood cancer, follow up, risk factor

Article summary

Article focus: (up to 3 bullets on the research questions or hypotheses addressed)

- There is limited information on the aetiology of childhood cancers and psychological stress may play a role.
- We investigated the association between psychological stress following bereavement by the death of a close relative early in life and subsequent childhood cancer.

Key messages: (up to 3 bullets on the key messages/significance)

- A small (10%) increase in the risk of childhood cancer was seen among those who experienced the death of a close relative.
- The association between early life stress and childhood cancer was small, but adds to our understanding of the causes of childhood cancers.

Strengths and limitations

- The study utilizes high quality nationwide registers in two countries. Large sample sizes are important for due to the rareness of childhood cancer.
- There are probably other sources of stress on which we do not have information, but the death of a close relative is considered to be one of the most stressful experiences, which will provide a large exposure contrast.
- The limited information on the aetiology of childhood cancers means that confounder control is incomplete.

Introduction

Childhood cancer is a leading cause of child deaths in affluent societies [1,2]. Almost half of childhood cancers are diagnosed before 5 years of age [2], highlighting the importance of identifying early life risk factors for developing prevention strategies [2,3] but, in comparison to adult cancers, known risk factors are few[4,5]. Additionally, heterogeneity and rareness of childhood cancers make investigation in populations challenging.

Studies in adults have reported increased cancer risks following psychological stress [6,7]. Psychological stress activates the nervous system and the hypothalamic-pituitary-adrenal axis, leading to release of hormones such as glucocorticoids and norepinephrine. Research has shown stress and the subsequent hormonal dysfunction can lead to impairment of DNA repair [8] and suppression of the immune response [9]. Additionally, stress may lead to epigenetic silencing: altering DNA methylation and histone acetylation [10], which are important during tumour development [11-13].

Little is known about the effect of stress in early postnatal life on the risk of childhood cancers and the effect of stress is potentially a much more complex exposure in children than in adults. First, children have immature body systems; growth and differentiation of their organs can be disrupted [14], potentially increasing susceptibility to environmental exposures. Through its effects on immune function, stress may increase susceptibility to infections, which have also been linked to certain childhood cancers [15-18]. However, “resilience to adversity” [19] might imply that stress may not lead to the same hormonal and immunological disturbances observed in adults. Our previous work on psychological stress in adults uses bereavement, considered to be one of the

most stressful experiences [20], as an indicator for psychological stress [6,21]. Just as adults may engage in risk behaviors following stress [22], children may be exposed to additional risks (such as passive smoking, poor diet, physical inactivity, or premature cessation of breast feeding) if parents altered their own lifestyle due to bereavement, which in turn impacts their children. However, the death of a relative may have further consequences for children. Other changes may also occur following bereavement, including reduced economic resources, changes in care [23] or a change in parents' ability to fulfill parental roles due to their own grief [24]. Another difference is that young children lack an understanding about the consequences of death [24], and therefore the experiences of psychological stress following bereavement may vary compared with adults and between age groups in childhood.

We investigated the association between bereavement in early life and the subsequent risk of childhood cancers. We hypothesized that that risks would be of a larger magnitude following the death of a close relative versus the death of other relatives, and sudden death versus other death [6,21]. In addition, we hypothesized that risks would vary with timing of the exposure, due to differences in awareness of loss [24] and susceptibility to changes in family structure [23,24].

Materials and Methods

Study participants and follow-up

This population-based cohort study used data from Danish and Swedish national registers and its design has been described elsewhere [25]. In brief, the unique civil personal registration number, which is assigned to all live-born children and new residents, was used to link children to their relatives and to information on birth, death, demographics, social data and various health outcomes from different national registers. Children of mothers with no personal registration number recorded were excluded as they could not be linked to their relatives (N=10,641; 0.17%). Additionally, children diagnosed with cancer within three months of birth were excluded, to remove cancers likely to have been prevalent at birth (N=348; 0.01%). The remaining study populations included 2,729,308 children from Denmark and 3,395,166 children from Sweden.

The exposure for the study is bereavement by the death of a close relative. Children born in Denmark from 1968 to 2007 and in Sweden from 1973 to 2006 were linked to their relatives (parents, siblings, mother's siblings and mother's parents) by their personal registration number, using the Danish Civil Registry System and the Swedish Multigeneration Register. Data on relatives' deaths were obtained from the Danish Civil Registry and the Swedish Cause of Death Registry. Follow up started at birth when all children were classified as 'unexposed'. Children would be categorized as 'exposed' when they experienced the death of a close relative, and afterwards contributed observation time for the exposed group. Follow up ended at the first of the following events: date of a cancer diagnosis, death, emigration, day before their 15th birthday or end of follow up (31st December 2006 in Sweden and 31st December 2007 in Denmark). Covariates were selected a priori according to previous literature: potential confounders at baseline (birth)

included maternal age (≤26 years, 27-30 years, >30 years), parity (1, 2, ≥3), multiplicity, maternal education level at birth (≤9 years, 10-14 years, >15 years), and maternal smoking during pregnancy. Data on covariates were obtained from the Medical Birth Registries, the Swedish Education Register and the Danish Integrated Database for Labour Market Research.

The data on all incident cancers (ICD-7 codes 104-205, ICD-10 codes C00-97) diagnosed up to the 15th year of age was obtained from the Swedish and Danish National Cancer Registries [26,27]. . Additionally, specific childhood cancer diagnoses previously suggested to be related to stress, hormones or immune status were considered, including testicular cancer (ICD-7 178, ICD-10 C62) [28], leukaemias (ICD-7 204, ICD-10 C91-95) [29,30] or lymphomas (Hodgkin's lymphoma (ICD-7 201, ICD-10 C81) and non-Hodgkin's lymphoma (ICD-7 200 and 202, ICD-10 C82-83)) [21,30,31]. As there is limited evidence on childhood cancer aetiology, we also included central nervous system (CNS) tumours (ICD-7 193, ICD-10 C70-72) and Wilms' tumour (ICD-7 180, ICD-10 C69.2).

Statistical analysis

Statistical analyses were carried out using Stata 11. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using the Cox regression model, accounting for some mothers having more than one child with robust estimation. Proportional-hazards assumption was evaluated using the *estat phtest* function, which tests the assumption on the basis of Schoenfeld residuals. The analyses were stratified by type of bereavement (the death of a parent/sibling, or of another relative: mother's parents or mother's siblings), cause of death (unexpected death due to an accident, suicide or violence, or other death) and timing of the exposure (age at exposure 0-1 years, 2-5 years, 6-9 years or 10-14 years) [20,32,33]. Potential confounders (country, maternal

age, parity and multiplicity) were adjusted for. Additionally, stratification was carried out by sex, country, birth weight, 5-minute Apgar score and gestational age of the children to assess for effect measure modification: children born with low or high birth weight, preterm with a low Apgar score may be more susceptible to suffer severe health consequences of hazardous exposures early in life, such as stress. In sub-analyses, we also adjusted for data on smoking during pregnancy (which were available from 1991 onwards in Denmark and from 1983 onwards in Sweden) and maternal education at child birth (available from the start of the data in Sweden (1973), but from 1980 onwards in Denmark). These were considered to be possible confounders, but were only available for limited periods and had a relatively high proportion of missing values. Therefore they were included only in sub-analyses to assess whether they altered the results. Birth year was added to adjust for calendar time. To check for the possibility of confounding by a genetic predisposition to cancer, the analyses were repeated following exclusion of children whose bereavement was caused by death of a parent from cancer. Additionally, analyses were carried out where children were moved to the exposed group three months and then 12 months after they experienced a death of a relative, to allow some time for a potential physiological effect of the bereavement. Finally, multiple imputations were carried out for missing covariates using Stata's *ice* command. Maternal origin, birth year, birth weight and gestational age were also included in the imputation model.

The study was approved by the Danish Data Protection Agency (j nr. 2008-41-2680), Scientific Ethics Committee of Central Jutland Region (VEK, sagnr. M-20100252) and the Research Ethic Committee (EPN) at the Karolinska Institute (Ref no. 2008/4:6).

Results

Of the 6,124,474 children, 1,505,938 (24.5%) experienced death of a relative during the follow up period of 71.9 million person years. In the cohort, 9823 children were diagnosed with cancer (incidence rate 13.7 per 100,000 person years); the most common being leukaemias (2882), cancers of the CNS (2546) and Wilms' tumours (606). Of children exposed to death of a relative, 1350 received a diagnosis of childhood cancer.

The characteristics of the study population are provided in Table 1. Children in the exposed group were more likely to have had low birth weight, to be of higher birth order and to be born to mothers who were older, of Nordic origin, with lower education levels and who more often reported smoking during pregnancy. Additionally, children in the exposed cohort were more likely to have a low Apgar score at five minutes (Table 1).

[INSERT TABLE 1 HERE]

The associations between bereavement and childhood cancer are displayed in Table 2. Compared with unexposed children, exposed children had a slightly increased cancer risk (HR: 1.10; 95% CI: 1.04-1.17). When stratifying by type of relative, the association between parent/sibling death and childhood cancers was positive, but statistically insignificant. For more distant relatives the association was smaller, but statistically significant. The association was also significant for who experienced the death of a relative due to a disease and for those bereaved between 2-5 years of age (Table 2). Adding birth year, maternal smoking during pregnancy and maternal education at birth to the models did not alter the results (data not shown).

[INSERT TABLE 2 HERE]

When we excluded children exposed to the death of a parent from cancer (n=31,737), the overall risk of childhood cancer related to loss of a relative was almost unchanged (HR: 1.09; 95% CI: 1.03-1.16); and if the death was due to a disease, the HR (95% CI) was 1.10 (1.04-1.18). Moving children to the exposed group three months (HR: 1.09; 95% CI: 1.03-1.16) or 12 months (HR: 1.08; 95% CI 1.01-1.16) after they experienced the death of a relative also gave similar results. Following imputation of missing data, results were not greatly altered: the HR (95% CI) was 1.13 (1.07-1.20).

Stratified analyses were carried out to assess for effect measure modification by sex, birth weight (low <2500g, normal 2500-3999g, high ≥4000g), gestational age (preterm <37weeks, term ≥37weeks) or 5-minute Apgar score (low <7, normal 7-10). The estimates for each strata did not differ significantly, so significant effect modification was not observed, but exposure groups became small for these analyses. Additionally, the data was stratified by country to see if the association was different in the two countries. While confidence intervals for the HRs overlapped, the association between exposure and childhood cancer was slightly larger for Sweden (HR: 1.12; 95% CI: 1.03-1.20) than for Denmark (HR: 1.07; 95% CI: 0.97-1.19).

The associations between bereavement and specific childhood cancers are shown in Table 3. Numbers of cases were particularly small for testicular cancer and Hodgkin's lymphoma, and for exposure subgroups; results from subgroups are therefore not displayed. Those exposed were observed to have a significantly increased risk for CNS tumours (HR: 1.14; 95% CI: 1.02-1.28). A positive association was also seen for leukemia (HR: 1.12; 95% CI: 1.00-1.26) (Table 3).

[INSERT TABLE 3 HERE]

Discussion

In this large population-based cohort study, a small but statistically significant overall increased risk of childhood cancer was observed among children exposed to bereavement due to the death of a family member. Exposure was also associated with CNS tumours and leukaemia. However, limited number of cases prevented us from obtaining informative estimates for most of specific childhood cancers. Whether this is causally related to the stress exposure or a consequence of other factors is unknown.

The main methodological strength of the study is the use of the large, longitudinal, nationwide registers from the Denmark and Sweden. Data was collected prospectively and is of high quality, with almost complete follow up [34]. Although childhood cancers are a leading cause of childhood death in affluent countries, they are not very common, making ad hoc follow up studies rare. The cancer registries have high levels of completeness [26,27]. Bereavement due to death of a relative is considered one of the most stressful life events [20], irrespective of coping style [35].

A limitation is the uncertainty of induction and latency periods for childhood cancer. The main analysis was therefore repeated twice considering start of exposure to be three months or one year after they experienced the bereavement, allowing some time for a potential physiological effect of the bereavement. This did not provide different results. Future research may suggest more evidence based lag times for childhood cancer research. Compared with the unexposed group, there were fewer missing values for some co-variates in the exposed group. This may partly be due to a higher proportion of children born to mothers of Nordic origin in the exposed group (98.5% compared with 94.0%), as information for mothers of non-Nordic origin may be less

complete. We did not have information on other stressful events, for example parental divorce or the death of a caregiver. However those who experienced other causes of stress would be categorized as 'unexposed', and such misclassification would probably draw risk estimates towards the null. Finally, despite a large sample size, case numbers for some specific cancers were small.

We have previously reported that mothers who experience the death of a child have increased cancer risk [6], and if mothers are exposed to bereavement during pregnancy, the risk of some childhood cancers in the offspring is increased [21]. Considering the differences in the effects of stress in children compared to adults, we hypothesized variation in the size of the association based on age at bereavement, as there may be little awareness of a loss at a very young age. Although a significant increase in risk was seen following bereavement between the ages of 2-5 years, there were no significant differences between age groups. One potential reason is that bereavement, particularly of a close relative, can be a long lasting stressor leading to allostatic load [36], and not limited to the period immediately after bereavement. Other studies looking at stressful life events and malignant disease in adults have produced mixed results: some found no association [37-42], while some have reported increased risks [6,7]. Most studies have included less severe stress exposures than loss of a close relative, and similar to our findings, positive associations have generally been of modest magnitude, indicating that if stress is a causal factor it is only one of many potential causes.

Although it is unlikely that bereavement increases risks for all childhood cancers, the observed associations suggest a role of for hormonal disturbance perhaps as a general promoter. The

function of immune cells following a stressful exposure may impair the ability to detect and deal with cancerous cells and eliminate infections. For example, in adults stress may inhibit apoptosis [9]. Bereavement during childhood has been described as a "tolerable stress", which can be overcome with the right support, but may become "toxic" if unmanaged [43]. Thus, insufficient coping and limited social support may further lead to allostatic load prolonging the hormonal imbalance. Whether the associations reflect a direct causal effect of stress or an indirect effect, mediated by other changes (for example, diet, infections or family dynamics), or are a result of unadjusted confounding is not known. We do not know more than a small fraction of childhood cancer causes, making confounder adjustments far from complete. We had expected that any observed increases in childhood cancer in the exposed group to be due to increases in cancers related to hormone and immune status. While an increase was seen for leukaemias, it was also seen for CNS tumours, which was not based on an a priori hypothesis. However,the 'two-hit theory' [44] and multi-step theory [45] of carcinogenesis suggest that at least two mutagenic hits are necessary for cancer development; research has suggested that some childhood cancers, including leukaemias [46] and CNS tumours [47], are initiated in utero. If psychological stress does affect childhood cancer risk, bereavement may act as or facilitate the second 'hit'. The need for exposure to a risk factor during gestation to initiate development of a childhood cancer and the vast number of other potential causes of 'hits' could explain the relatively small association seen in this study, for cancers which have been suggested to be initiated in utero.

If stress has a causal relation with certain childhood cancers, it would be expected to vary with the intensity of stress. However, stress is a "highly individualistic experience" [48], which may make it difficult to consider a dose-response effect. The risk of childhood cancer was not higher in those

who lost a parent or a sibling than in those who lost a more distant relative. Additionally, there were no differences in risk if the loss was sudden or due to a disease, but numbers become small for these sub-analyses. It is also difficult to hypothesize, especially in this age group, which would cause a greater level of stress: an unexpected loss or loss from a chronic disease. A long term effect may be more important, which follow either type of loss.

Our data suggests that psychological stress in early life is associated with an increased risk of some childhood cancers. Early life bereavement may also have long term effects on cancer risk. For example, epigenetic changes or impairment of DNA repair may reduce the body's ability to deal with the future carcinogenic exposures [8,12,13]. Inclusion of data from more countries or over a longer time period could provide greater power to better assess the association between stress and specific cancers. The association between early life stress and childhood cancer was small, but adds to our general understanding of the causes and development of childhood cancers.

Contributors: JL and JO conceived the research. NM analysed the data and wrote the first draft of the manuscript. NM, JO, SC, MG and JL contributed to data analysis, interpretation of results and critical revision of the manuscript.

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Competing interests: The authors disclose no potential conflicts of interest

Data sharing: The data is accessed via secure server at Statistics Denmark. We, the researchers, are unable to provide access to the raw data.

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Table 1. Descriptive statistics of the study population

	Exposed cohort (N = 1,426,013)	Unexposed cohort (N = 6,123,531)	P value
Variables	**N (%)**	**N (%)**	
Sex			
Male	773,690 (51)	3,143,231 (51)	
Female	732,244 (49)	2,980,280 (49)	
Missing	4 (<1)	20 (<1)	0.564
Birth weight [a]			
<2500g	68,125 (5)	232,387 (4)	
2500g-3999g	1,119,223 (77)	4,024,314 (75)	
≥4000g	250,538 (17)	914,659 (17)	
Missing	22,344 (2)	215,793 (4)	<0.01
Maternal age			
≤26	519,495 (35)	2,398,883 (39)	
27-30	443,592 (30)	1,777,990 (29)	
≥31	542,782 (36)	1,946,022 (32)	
Missing	69 (<1)	636 (<1)	<0.01
Gestational age [a]			
<37 weeks	82,407 (6)	82,407 (6)	
≥37 weeks	1,351,860 (93)	1,351,860 (93)	
Missing	25,961 (2)	25,961 (2)	<0.01
Parity [b]			
1	582,558 (41)	2,648,077 (46)	
2	519,142 (37)	2,030,259 (35)	
≥3	305,963 (22)	1,059,912 (18)	
Missing	806 (<1)	10,633 (<1)	<0.01
Maternal education [c]			
Low, ≤9 years	701,579 (49)	2,306,817 (44)	
Middle, 10-14 years	398,797 (28)	1,461,584 (28)	
High, ≥15 years	296,247 (21)	1,046,192 (20)	
Missing	44,454 (3)	442,534 (8)	<0.01
Maternal smoking during pregnancy [d]			
Yes	222.980 (26)	703,266 (19)	
No	572.800 (66)	2,674,088 (72)	
Missing	69,925 (8)	315,934 (9)	<0.01
Singleton [b]			
Yes	1,437,016 (97)	5,451,148 (95)	
No	39,129 (3)	147,173 (3)	
Missing	10,030 (1)	150,560 (3)	<0.01
Apgar score at 5 minutes [a]			
Low, 0-6	13,960 (1)	52,667 (1)	
Normal, 7-10	1,352,106 (93)	4,946,721 (92)	
Missing	94,162 (6)	387,762 (7)	<0.01
Country			
Denmark	663,820 (44)	2,728,862 (45)	
Sweden	842,118 (56)	3,394,669 (55)	<0.01
Maternal origin			

Nordic	1,482,681 (99)	5,758,361 (94)	
Non-Nordic	19,532 (1)	333,804 (6)	
Missing	3,725 (<1)	31,366 (1)	<0.01

[a] available from 1978 in Denmark and from 1973 in Sweden; [b] available from 1973 in Denmark and from 1973 in Sweden; [c] available from 1980 in Denmark and from 1973 in Sweden; [d] available from 1991 in Denmark and from 1983 in Sweden

Table 2. Hazard ratios (HR) for all childhood cancers according to exposure status

Bereavement	Cancer cases (rate 1/100 000 person years)	Crude HR	Adjusted HR (95% CI) [a]
All exposed	1350 (13.88)	1.15	1.10 (1.04-1.17)
Type of deceased relative			
Parent/sibling	140 (14.44)	1.20	1.18 (0.99-1.41)
Other relatives	1210 (13.82)	1.14	1.09 (1.03-1.16)
Cause of death			
Unexpected	132 (12.83)	1.06	1.03 (0.87-1.23)
Other	1217 (14.07)	1.16	1.11 (1.04-1.19)
Age at exposure			
0-1 years	350 (14.88)	1.10	1.06 (0.95-1.18)
2-5 years	536 (13.88)	1.17	1.13 (1.03-1.23)
6-9 years	306 (12.48)	1.13	1.08 (0.95-1.21)
10-14 years	158 (14.88)	1.17	1.14 (0.99-1.39)
Unexposed	8473 (13.64)	1.0 (ref)	1.0 (ref)

[a] Adjusted for country, maternal characteristics at birth (maternal age and parity) and whether child was a twin. Reduced to 9382 failures (and 9381 for unexpected vs other) due to missing values for covariates and twin/singleton data availability from 1973.

Table 3. Hazard ratios (HR) for specific childhood cancers according to bereavement

Cancer		Cases (rate per 100,000 person years)	Crude HR	Adjusted HR (95% CI) [a]
Leukaemias	Unexposed	2522 (4.06)	1.0 (ref)	1.0 (ref)
	All Exposed	360 (3.70)	1.2	1.12 (1.00-1.26)
Hodgkin's Lymphoma	Unexposed	123 (0.20)	1.0 (ref)	1.0 (ref)
	All Exposed	43 (0.44)	1.29	1.17 (0.81-1.67)
Non-Hodgkin's Lymphoma	Unexposed	441 (0.71)	1.0 (ref)	1.0 (ref)
	All Exposed	68 (0.70)	1.03	0.99 (0.76-1.29)
CNS tumours	Unexposed	2160 (3.48)	1.0 (ref)	1.0 (ref)
	All Exposed	386 (3.97)	1.19	1.14 (1.02-1.28)
Wilms' tumour [b]	Unexposed	564 (0.91)	1.0 (ref)	1.0 (ref)
	All Exposed	42 (0.43)	0.98	0.93 (0.68-1.28)
Testicular cancer	Unexposed	50 (0.08)	1.0 (ref)	1.0 (ref)
	All Exposed	5 (0.05)	1.16	1.08 (0.41-2.88)

[a] Adjusted for country, maternal characteristics at birth (maternal age and parity) and whether child was a twin.
[b] Proportional hazards assumption not met by model for this cancer subtype.

Title: Early life bereavement and childhood cancer: a nationwide follow-up study in two countries

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Key words: bereavement, psychological stress, childhood cancer, risk factor , follow up

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Abstract

Objective: Childhood cancer is a leading cause of child deaths in affluent countries but little is known about its aetiology. Psychological stress has been suggested to be associated with cancer in adults; whether this is also seen in childhood cancer is largely unknown. We investigated the association between bereavement as an indicator of severe childhood stress exposure and childhood cancer, using data from Danish and Swedish national registers.

Design: Population-based cohort study.

Setting: Denmark and Sweden.

Participants: All live-born children born in Denmark between 1968 and 2007 (N=2,729,308) and in Sweden between 1973 and 2006 (N=3,395,166) were included in this study. Exposure was bereavement by the death of a close relative before 15 years of age. Follow up started from birth and ended at the first of the following: date of a cancer diagnosis, death, emigration, day before their 15th birthday or end of follow up (2007 in Denmark, 2006 in Sweden).

Outcome measures: Rates and hazard ratios for all childhood cancers and specific childhood cancers.

Results: A total of 1,505,938 (24.5%) children experienced bereavement at some point during their childhood and ~~. There were~~ 9823 ~~who received a cancer diagnosis~~were diagnosed with cancer before the age of 15 years. Exposed children had a small (10%) increased risk of childhood cancer (hazard ratio (HR): 1.10; 95% confidence interval (CI) 1.04-1.17). For specific cancers, a significant association was seen only for central nervous system tumours (HR: 1.14; 95% CI: 1.02-1.28).

Conclusions: Our data suggests psychological stress in early life is associated with a small increased risk of childhood cancer.

Key words: bereavement, psychological stress, childhood cancer, follow up, risk factor

Article summary

Article focus: (up to 3 bullets on the research questions or hypotheses addressed)

- There is limited information on the aetiology of childhood cancers and psychological stress may play a role.
- We investigated the association between psychological stress following bereavement by the death of a close relative early in life and subsequent childhood cancer.

Key messages: (up to 3 bullets on the key messages/significance)

- A small (10%) increase in the risk of childhood cancer was seen among those who experienced the death of a close relative.
- The association between early life stress and childhood cancer was small, but adds to our understanding of the causes of childhood cancers.

Strengths and limitations

- The study utilizes high quality nationwide registers in two countries~~, which contain data of high quality~~. ~~Such a l~~Large sample size~~s~~ ~~is~~are important for ~~such research~~ due to the rareness of childhood cancer.
- There are probably other sources of stress on which we do not have information, but the death of a close relative is considered to be one of the most stressful experiences, which will provide a ~~good~~ large exposure contrast.
- The limited information on the aetiology of childhood cancers means that confounder control ~~may be~~is incomplete~~ in this study~~.

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Introduction

Childhood cancer is a leading cause of child deaths in affluent societies [1,2]. Almost half of childhood cancers are diagnosed before 5 years of age [2], highlighting the importance of identifying early life risk factors for developing prevention strategies [2,3] but, in comparison to adult cancers, known risk factors are few~~, except for radiation~~ [4,5]. Additionally, heterogeneity and rareness of childhood cancers make investigation in populations challenging.

Studies in adults have reported increased cancer risks following psychological stress [6,7]. Psychological stress activates the nervous system and the hypothalamic-pituitary-adrenal axis, leading to release of hormones such as glucocorticoids and norepinephrine. Research has shown stress and the subsequent hormonal dysfunction can lead to impairment of DNA repair [8] and suppression of the immune response [9]. Additionally, stress may lead to epigenetic silencing: altering DNA methylation and histone acetylation [10], which are important during tumour development [11-13]~~[11,12]~~.

Little is known~~, however,~~ about the effect of stress in early postnatal life on the risk of childhood cancers and the effect of stress is potentially a much more complex exposure in children than in adults. First~~ly~~, children have immature body systems; growth and differentiation of their organs can be disrupted [14]~~[13]~~, potentially increasing susceptibility to environmental exposures. Through its effects on immune function, stress may increase susceptibility to infections, which have also been linked to certain childhood cancers [15-18]~~[14-17]~~. However, “resilience to adversity” [19]~~[18]~~ might imply that stress may not lead to the same hormonal and immunological disturbances observed in adults. Our previous work on psychological stress in adults uses

bereavement, considered to be one of the most stressful experiences [20]~~[19]~~, as an indicator for psychological stress [6,21]~~[6,20]~~. Just as adults may engage in risk behaviors following stress [22]~~[21]~~, children may be exposed to additional risks (such as passive smoking, poor diet, physical inactivity, or premature cessation of breast feeding) if parents altered their own lifestyle due to bereavement, which in turn impacts their children. However, the death of a relative may have further consequences for children. Other changes may also occur following bereavement, including reduced economic resources, changes in care [23]~~[22]~~ or a change in parents' ability to fulfill parental roles due to their own grief [24]~~[23]~~. Another difference is that young children ~~may a~~ lack ~~of~~ an understanding about the consequences of death [24]~~[23]~~, and therefore the experiences of psychological stress following bereavement may vary compared with adults and between age groups in childhood.

We investigated the association between bereavement in early life and the subsequent risk of childhood cancers. We hypothesized that that risks would be of a larger magnitude following the death of a close relative versus the death of other relatives, and sudden death versus other death [6,21]~~[6,20]~~. In addition, we hypothesized that risks would vary with timing of the exposure, due to differences in awareness of loss [24]~~[23]~~ and susceptibility to changes in family structure [23,24]~~[22,23]~~.

Materials and Methods

Study participants and follow-up

This population-based cohort study used data from Danish and Swedish national registers and its design has been described elsewhere [25]~~[24]~~. In brief, the unique civil personal registration number, which is assigned to all live-born children and new residents, was used to link children to their relatives and to information on birth, death, demographics, social data and various health outcomes from different national registers. Children of mothers with no personal registration number recorded were excluded as they could not be linked to their relatives (N=10,641; 0.17%). Additionally, children diagnosed with cancer within three months of birth were excluded, to remove cancers likely to have been prevalent at birth (N=348; 0.01%). The remaining study populations included 2,729,308 children from Denmark and 3,395,166 children from Sweden.

The exposure for the study is bereavement by the death of a close relative. Children born in Denmark from 1968 to 2007 and in Sweden from 1973 to 2006 were linked to their relatives (parents, siblings, mother's siblings and mother's parents) by their personal registration number, using the Danish Civil Registry System and the Swedish Multigeneration Register. Data on relatives' deaths were obtained from the Danish Civil Registry and the Swedish Cause of Death Registry. Follow up started at birth when all children were classified as 'unexposed'. Children would be categorized as 'exposed' when they experienced the death of a close relative, and afterwards contributed observation time for the exposed group. Follow up ended at the first of the following events: date of a cancer diagnosis, death, emigration, day before their 15th birthday or end of follow up (31st December 2006 in Sweden and 31st December 2007 in Denmark). Covariates were selected a priori according to previous literature: potential confounders at baseline (birth)

included maternal age (≤26 years, 27-30 years, >30 years), parity (1, 2, ≥3), multiplicity, maternal education level at birth (≤9 years, 10-14 years, >154 years), and maternal smoking during pregnancy. Data on covariates were obtained from the Medical Birth Registries, the Swedish Education Register and the Danish Integrated Database for Labour Market Research.

The data on all incident cancers (ICD-7 codes 104-205, ICD-10 codes C00-97) diagnosed up to the 15th year of age ~~cancer diagnoses~~ was obtained from the Swedish and Danish National Cancer Registries [26,27]~~[25,26]~~. ~~The main outcomes of interest were all incident cancers (ICD-7 codes 104-205, ICD-10 codes C00-97) diagnosed up to the 15th year of age~~. Additionally, specific childhood cancer diagnoses previously suggested to be related to stress, hormones or immune status were considered, including testicular cancer (ICD-7 178, ICD-10 C62) [28]~~[27]~~, leukaemias (ICD-7 204, ICD-10 C91-95) [29,30]~~[28,29]~~ or lymphomas (Hodgkin's lymphoma (ICD-7 201, ICD-10 C81) and non-Hodgkin's lymphoma (ICD-7 200 and 202, ICD-10 C82-83)) [21,30,31]~~[20,29,30]~~. As there is limited evidence on childhood cancer aetiology, we also included central nervous system (CNS) tumours (ICD-7 193, ICD-10 C70-72) and Wilms' tumour (ICD-7 180, ICD-10 C69.2).

Statistical analysis

Statistical analyses were carried out using Stata 11. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using the Cox regression model, accounting for some mothers having more than one child ~~in the cohort by using~~with robust estimation. Proportional-hazards assumption was evaluated using the *estat phtest* function, which tests the assumption on the basis of Schoenfeld residuals. The analyses were stratified by type of bereavement (the death of a parent/sibling, or of another relative: mother's parents or mother's siblings), cause of death

(unexpected death due to an accident, suicide or violence, or other death) and timing of the exposure (age at exposure 0-1 years, 2-5 years, 6-9 years or 10-14 years) [20,32,33]~~[19,31,32]~~. Potential confounders ~~like~~(country, -maternal age, parity and multiplicity) were adjusted for. Additionally, stratification was carried out by sex, country, birth weight, 5-minute Apgar score and gestational age of the children to assess for effect measure modification:~~.~~ children born with low or high birth weight, preterm with a low Apgar score may be more susceptible to suffer severe health consequences of hazardous exposures early in life, such as stress. In sub-analyses, we also adjusted for data on smoking during pregnancy (which were available from 1991 onwards in Denmark and from 1983 onwards in Sweden) and maternal education at child birth (available from the start of the data in Sweden (1973), but from 1980 onwards in Denmark~~ and from 1973 onwards in Sweden~~). These were considered to be possible confounders, but were only available for limited periods and had a relatively high proportion of missing values. Therefore they were included only in sub-analyses to assess whether they altered the results.~~,~~ ~~and b~~Birth year was added to adjust for calendar time. To check for the possibility of confounding by a genetic predisposition to cancer, the analyses were repeated following exclusion of children whose bereavement was caused by death of a parent from cancer. Additionally, analyses were carried out where children were moved to the exposed group three months and then 12 months after they experienced a death of a relative, to allow some time for a potential physiological effect of the bereavement. Finally, multiple imputations were carried out for missing covariates using Stata's *ice* command. Maternal origin, birth year, birth weight and gestational age were also included in the imputation model.

The study was approved by the Danish Data Protection Agency (j nr. 2008-41-2680), Scientific Ethics Committee of Central Jutland Region (VEK, sagnr. M-20100252) and the Research Ethic Committee (EPN) at the Karolinska Institute (Ref no. 2008/4:6).

Results

Of the 6,124,474 children, 1,505,938 (24.5%) experienced death of a relative during the follow up period of 71.9 million person years. In the cohort, 9823 children were diagnosed with cancer (incidence rate 13.7 per 100,000 person years); the most common being leukaemias (2882), cancers of the CNS (2546) and Wilms' tumours (606). Of children exposed to death of a relative, 1350 received a diagnosis of childhood cancer.

The characteristics of the study population are provided in Table 1. Children in the exposed group were more likely to have had low birth weight, to be of higher birth order and to be born to mothers who were older, of Nordic origin, with lower education levels and who more often reported smoking during pregnancy. Additionally, children in the exposed cohort were more likely to have a low Apgar score at five minutes (Table 1).

[INSERT TABLE 1 HERE]

The associations between bereavement and childhood cancer are displayed in Table 2. Compared with unexposed children, exposed children had a slightly increased cancer risk (HR: 1.10; 95% CI: 1.04-1.17). When ~~splitting~~ stratifying by type of relative, the association between parent/sibling death and childhood cancers was positive, but statistically insignificant. For more distant relatives the association was smaller, but statistically significant. The association was also significant for who experienced the death of a relative due to a disease and for those bereaved between 2-5 years of age (Table 2). Adding birth year, maternal smoking during pregnancy and maternal education at birth to the models did not alter the results (data not shown).

[INSERT TABLE 2 HERE]

When we excluded children ~~whose exposure status changed following~~exposed to the death of a parent from cancer (n=31,737), the overall risk of childhood cancer related to loss of a relative was almost unchanged (HR: 1.09; 95% CI: 1.03-1.16); and if the death was due to a disease, the HR (95% CI) was 1.10 (1.04-1.18). Moving children to the exposed group three months ~~after they experienced a death of a relative also gave similar results~~ (HR: 1.09; 95% CI: 1.03-1.16) or 12 months (HR: 1.08; 95% CI 1.01-1.16) after they experienced the death of a relative also gave similar results. Following imputation of missing data, results were not greatly altered: the HR (95% CI) was 1.13 (1.07-1.20).

Stratified analyses were carried out to assess for effect measure modification by sex, birth weight (low <2500g, normal 2500-3999g, high ≥4000g), gestational age (preterm <37weeks, term ≥37weeks) or 5-minute Apgar score (low <7, normal 7-10).~~Stratified analyses did not show significant effect measure modification by sex, low (<2500g) versus normal (≥2500g) birth weight and preterm (<37 weeks) versus term (≥37 weeks) birth (data not displayed),~~ The estimates for each strata did not differ significantly, so significant effect modification was not observed, but exposure groups became small for these analyses. Additionally, the data was stratified by country to see if the association was different in the two countries ~~(Sweden versus Denmark)~~. While confidence intervals for the HRs overlapped, the association between exposure and childhood cancer was slightly larger for Sweden (HR: 1.12; 95% CI: 1.03-1.20) than for Denmark (HR: 1.07; 95% CI: 0.97-1.19).

The associations between bereavement and specific childhood cancers are shown in Table 3. Numbers of cases were particularly small for testicular cancer and Hodgkin's lymphoma, and for exposure subgroups; results from subgroups are therefore not displayed. Those exposed were observed to have a significantly increased risk for CNS tumours (HR: 1.14; 95% CI: 1.02-1.28). A positive association was also seen for leukemia (HR: 1.12; 95% CI: 1.00-1.26) (Table 3).

[INSERT TABLE 3 HERE]

Discussion

In this large population-based cohort study, a small but statistically significant overall increased risk of childhood cancer was observed among children exposed to bereavement due to the death of a family member. Exposure was also associated with CNS tumours and leukaemia. However, limited number of cases prevented us from obtaining informative estimates for most of specific childhood cancers. Whether this is causally related to the stress exposure or a consequence of other factors is unknown.

The main methodological strength of the study is the use of the large, longitudinal, nationwide registers from the Denmark and Sweden. Data was collected prospectively and is of high quality, with almost complete follow up [34]~~[33]~~. Although childhood cancers are a leading cause of childhood death in affluent countries, they are not very common, making ad hoc follow up studies rare ~~in previous literature~~. The cancer registries have high levels of completeness [26,27]~~[25,26]~~. Bereavement due to death of a relative ~~provides a good indicator of stress, which~~ is considered one of the most stressful life events [20]~~[19]~~, irrespective of coping style [35]~~[34]~~.

A limitation is the uncertainty of induction and latency periods for childhood cancer. The main analysis was therefore repeated twice ~~after~~ considering start of exposure to be three months or one year after they experienced the bereavement, allowing some time for a potential physiological effect of the bereavement. This did not provide different results. Future research may suggest more evidence based lag times for childhood cancer research. Compared with the unexposed group, there were fewer missing values for some co-variates in the exposed group. This may partly be due to a higher proportion of children born to mothers of Nordic origin in the

exposed group (98.5% compared with 94.0%), as information for mothers of non-Nordic origin may be less complete. We did not have information on other stressful events, for example parental divorce or the death of a caregiver. However those who experienced other causes of stress would be categorized as 'unexposed', and such misclassification would probably draw risk estimates towards the null~~.~~. Finally, despite a large sample size, case numbers for some specific cancers were small.

We have previously reported that mothers who experience the death of a child have increased cancer risk [6], and if mothers are exposed to bereavement during pregnancy, the risk of some childhood cancers in the offspring is increased [21]~~[20]~~. Considering the differences in the effects of stress in children compared to adults, we hypothesized variation in the size of the association based on age at bereavement, as there may be little awareness of a loss at a very young age. Although a significant increase in risk was seen following bereavement between the ages of 2-5 years, there were no significant differences between age groups. One potential reason is that bereavement, particularly of a close relative, can be a long lasting stressor leading to allostatic load [36]~~[35]~~, and not limited to the period immediately after bereavement. Other studies looking at stressful life events and malignant disease in adults have produced mixed results: some found no association [37-42]~~[36-41]~~, while some have reported increased risks [6,7]. Most studies have included less severe stress exposures than loss of a close relative, and similar to our findings, positive associations have generally been of modest magnitude, indicating that if stress is a causal factor it is only one of many potential causes.

Although it is unlikely that bereavement increases risks for all childhood cancers, the observed associations suggest a role of for hormonal disturbance perhaps as a general promoter. The function of immune cells following a stressful exposure may impair the ability to detect and deal with cancerous cells and eliminate infections. For example, in adults stress may inhibit apoptosis [9]. Bereavement during childhood has been described as a "tolerable stress", which can be overcome with the right support, but may become "toxic" if unmanaged [43]~~[42]~~. Thus, insufficient coping and limited social support may further lead to allostatic load prolonging the hormonal imbalance. Whether the associations reflect a direct causal effect of stress or an indirect effect, mediated by other changes (for example, diet, infections or family dynamics), or are a result of unadjusted confounding is not known. We do not know more than a small fraction of childhood cancer causes, making confounder adjustments far from complete. We had expected that any observed increases in childhood cancer in the exposed group to be due to increases in cancers related to hormone and immune status. While an increase was seen for leukaemias, it was also seen for CNS tumours, which was not based on an a priori hypothesis. However, ~~T~~the 'two-hit theory' [44]~~[43]~~ and multi-step theory [45]~~[44]~~ of carcinogenesis suggest that at least two mutagenic hits are necessary for cancer development;~~.~~ ~~R~~research has suggested that some childhood cancers, including leukaemias [46]~~[45]~~ and CNS tumours [47]~~[46]~~, are initiated in utero. If psychological stress does affect childhood cancer risk, bereavement may act as or facilitate the second 'hit'. The need for exposure to a risk factor during gestation to initiate development of a childhood cancer and the vast number of other potential causes of 'hits' could explain the relatively small association seen in this study, for cancers which have been suggested to be initiated in utero.

If stress has a causal relation with certain childhood cancers, it would be expected to vary with the intensity of stress. However, stress is a "highly individualistic experience" [48]~~[47]~~, which may make it difficult to consider a dose-response effect. The risk of childhood cancer was not higher in those who lost a parent or a sibling than in those who lost a more distant relative. Additionally, there were no differences in risk if the loss was sudden or due to a disease, but numbers become small for these sub-analyses. It is also difficult to hypothesize, especially in this age group, which would cause a greater level of stress: an unexpected loss or loss from a chronic disease. A long term effect may be more important, which follow either type of loss.

Our data suggests that psychological stress in early life is associated with an increased risk of some childhood cancers. Early life bereavement may also have long term effects on cancer risk. For example, epigenetic changes or impairment of DNA repair may reduce the body's ability to deal with the future carcinogenic exposures [8,12,13]~~[8,12]~~. Inclusion of data from more countries or over a longer time period could provide greater power to better assess the association between stress and specific cancers. The association between early life stress and childhood cancer was small, but adds to our general understanding of the causes and development of childhood cancers.

Contributors: JL and JO conceived the research. NM analysed the data and wrote the first draft of the manuscript. NM, JO, SC, MG and JL contributed to data analysis, interpretation of results and critical revision of the manuscript.

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Table 1. Descriptive statistics of the study population

	Exposed cohort (N = 1,426,013)	Unexposed cohort (N = 6,123,531)	P value
Variables	**N (%)**	**N (%)**	
Sex			
Male	773,690 (51)	3,143,231 (51)	
Female	732,244 (49)	2,980,280 (49)	
Missing	4 (<1)	20 (<1)	0.564
Birth weight [a]			
<2500g	68,125 (5)	232,387 (4)	
≥2500g-3999g	~~1,369,759~~ 1,119,223 (~~94~~77)	~~4,938,970~~4,024,314 (75~~92~~)	
≥4000g	250,538 (17)	914,659 (17)	
Missing	22,344 (2)	215,793 (4)	<0.01
Maternal age			
≤26	519,495 (35)	2,398,883 (39)	
27-30	443,592 (30)	1,777,990 (29)	
≥31	542,782 (36)	1,946,022 (32)	
Missing	69 (<1)	636 (<1)	<0.01
Gestational age [a]			
<37 weeks	82,407 (6)	82,407 (6)	
≥37 weeks	1,351,860 (93)	1,351,860 (93)	
Missing	25,961 (2)	25,961 (2)	<0.01
Parity [b]			
1	582,558 (41)	2,648,077 (46)	
2	519,142 (37)	2,030,259 (35)	
≥3	305,963 (22)	1,059,912 (18)	
Missing	806 (<1)	10,633 (<1)	<0.01
Maternal education [c]			
Low, ≤9 years	701,579 (49)	2,306,817 (44)	
Middle, 10-14 years	398,797 (28)	1,461,584 (28)	
High, ≥15 years	296,247 (21)	1,046,192 (20)	
Missing	44,454 (3)	442,534 (8)	<0.01
Maternal smoking during pregnancy [d]			
Yes	222.980 (26)	703,266 (19)	
No	572.800 (66)	2,674,088 (72)	
Missing	69,925 (8)	315,934 (9)	<0.01
Singleton [b]			
Yes	1,437,016 (97)	5,451,148 (95)	
No	39,129 (3)	147,173 (3)	
Missing	10,030 (1)	150,560 (3)	<0.01
Apgar score at 5 minutes [a]			
Low, 0-6	13,960 (1)	52,667 (1)	
Normal, 7-10	1,352,106 (93)	4,946,721 (92)	
Missing	94,162 (6)	387,762 (7)	<0.01
Country			
Denmark	663,820 (44)	2,728,862 (45)	
Sweden	842,118 (56)	3,394,669 (55)	<0.01
Maternal origin			

Nordic	1,482,681 (99)	5,758,361 (94)	
Non-Nordic	19,532 (1)	333,804 (6)	
Missing	3,725 (<1)	31,366 (1)	<0.01

[a] available from 1978 in Denmark and from 1973 in Sweden; [b] available from 1973 in Denmark and from 1973 in Sweden; [c] available from 1980 in Denmark and from 1973 in Sweden; [d] available from 1991 in Denmark and from 1983 in Sweden

Table 2. Hazard ratios (HR) for all childhood cancers according to exposure status

Bereavement	Cancer cases (rate 1/100 000 person years)	Crude HR	Adjusted HR (95% CI) [a]
All exposed	1350 (13.88)	1.15	1.10 (1.04-1.17)
Type of deceased relative			
Parent/sibling	140 (14.44)	1.20	1.18 (0.99-1.41)
Other relatives	1210 (13.82)	1.14	1.09 (1.03-1.16)
Cause of death			
Unexpected	132 (12.83)	1.06	1.03 (0.87-1.23)
Other	1217 (14.07)	1.16	1.11 (1.04-1.19)
Age at exposure			
0-1 years	350 (14.88)	1.10	1.06 (0.95-1.18)
2-5 years	536 (13.88)	1.17	1.13 (1.03-1.23)
6-9 years	306 (12.48)	1.13	1.08 (0.95-1.21)
10-14 years	158 (14.88)	1.17	1.14 (0.99-1.39)
Unexposed	8473 (13.64)	1.0 (ref)	1.0 (ref)

[a] Adjusted for country, maternal characteristics at birth (maternal age and parity) and whether child was a twin. Reduced to 9382 failures (and 9381 for unexpected vs other) due to missing values for covariates and twin/singleton data availability from 1973.

Table 3. Hazard ratios (HR) for specific childhood cancers according to bereavement

Cancer		Cases (rate per 100,000 person years)	Crude HR	Adjusted HR (95% CI) [a]
Leukaemias	Unexposed	2522 (4.06)	1.0 (ref)	1.0 (ref)
	All Exposed	360 (3.70)	1.2	1.12 (1.00-1.26)
Hodgkin's Lymphoma	Unexposed	123 (0.20)	1.0 (ref)	1.0 (ref)
	All Exposed	43 (0.44)	1.29	1.17 (0.81-1.67)
Non-Hodgkin's Lymphoma	Unexposed	441 (0.71)	1.0 (ref)	1.0 (ref)
	All Exposed	68 (0.70)	1.03	0.99 (0.76-1.29)
CNS tumours	Unexposed	2160 (3.48)	1.0 (ref)	1.0 (ref)
	All Exposed	386 (3.97)	1.19	1.14 (1.02-1.28)
Wilms' tumour [b]	Unexposed	564 (0.91)	1.0 (ref)	1.0 (ref)
	All Exposed	42 (0.43)	0.98	0.93 (0.68-1.28)
Testicular cancer	Unexposed	50 (0.08)	1.0 (ref)	1.0 (ref)
	All Exposed	5 (0.05)	1.16	1.08 (0.41-2.88)

[a] Adjusted for country, maternal characteristics at birth (maternal age and parity) and whether child was a twin.
[b] Proportional hazards assumption not met by model for this cancer subtype.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9
Bias	9	Describe any efforts to address potential sources of bias	8-9
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	8-9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10, Table 2
		(*b*) Report category boundaries when continuous variables were categorized	8, Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11, Table 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14-15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	4

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.